E-MODUL PRACTICAL GUIDE BOOK

SECONDARY METABOLITE OF NATURAL INGREDIENTS

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PREFACE

Thanks to Allah SWT. for His blessings and grace so we can write this practical guide book of secondary metabolites of natural ingredients. This book aims to provide students with understanding and skills regarding procedures for obtaining secondary metabolites.

Matters in this practicum include phytochemical screening, extraction, fractionation and isolation of active substances from medicinal plants. Phytochemical screening is carried out to identify the chemical group of a medicinal plant. Identification was carried out by color reaction, precipitation and thin layer chromatography. The extraction method used maceration and percolation. Isolation was carried out by fractionation with column chromatography and direct crystallization of the active substance from the extract.

Finally, we realize that this book is far from perfect. We really appreciate suggestions and constructive criticism from Pharmacist colleagues working in the field of natural ingredients and other related sciences for the perfection of this book.

Bengkulu , August 2022

Editor

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LABORATORY REGULATIONS

1. Student must enter laboratory appropriate time in accordance with each other's schedule. Lateness tolerated if the reason is can be tolerated.
2. Student moment enter room laboratory must already ready with lab coat, book practical guide book, book, stationary and other tools used for practicum.
3. Before practice, there will be pretest and discussion were carried out for test readiness student in follow practicum.
4. Every time one practical theory is done, student required make report while and ask approval (acc) of lecturer or assistant student who on duty.
5. Students who destroy, solve or remove equipment practice must report to tutor or technician laboratory and mandatory replace with the same quality.
6. Every student must make report collected practice - to the practicum mentor at the a week latest after relevant practicum done.
7. Student required guard cleanliness and tidiness table practice as well as bottles reagent.
8. Practical value consists from:

Score pretest	: 20 %
Value of reports and discussions	: 30 %
Practicum exam score	: 50 %

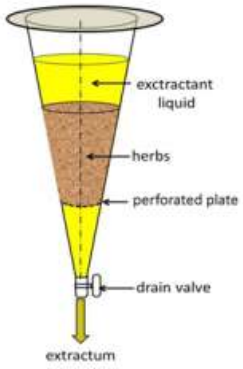
Bengkulu , August 2022

Compiler

INTRODUCTION TO PRACTICUM TOOLS

1. **Percolator**

   Percolator is a tool used to extract simplicia powder by flowing the extractor liquid through the powder which has been moistened with the solvent first. For clearer explanation please click the following link
   https://www.youtube.com/watch?v=q0iO0wqBwc0



(Julianto , 2019).

2. **Rotating Vaporizer (*Rotary evaporators*)**

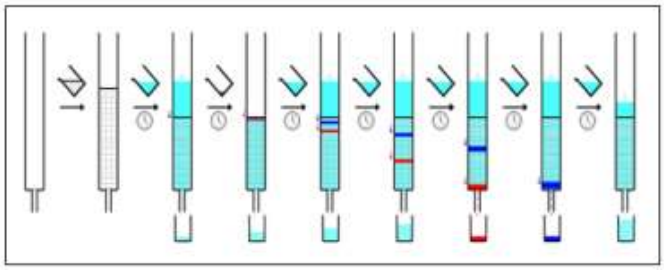
   A rotary evaporator is used to separate or evaporate the liquid extract from the material being extracted so that a concentrated extract is obtained. With this tool the process of evaporation of the filter liquid occurs by lowering its boiling point by lowering the pressure. Pictures can be seen below. Please click the following link :
   https://www.youtube.com/watch?v=APK-Ft6SFc8
   https://www.youtube.com/watch?v=yJ9xcrWiYSM

(Santoso *et al.,* 2021).

3. **Chromatography Column**

This tool is used to separate the active substance from other components using the principle of chromatography, namely the separation of a mixture of components based on the differences in the migration of these components from the stationary phase under the influence of the mobile phase. The column is a glass tube of a certain diameter with a diverter at the bottom. Pictures and their parts can be seen in the picture below (Markham, 1982) . Please click the following link

https://www.youtube.com/watch?v=lasQRWREStY
https://www.youtube.com/watch?v=AZQlTgeJcYA ,
https://www.youtube.com/watch?v=9cRpfJ0UiM0



(nurdiani, 2018).

4. **Gunshot Micro**

This tool is used to spot a certain amount of extract on the TLC plate. There are several sizes, such as 1 µL, 2 µL, and 5 µL.

https://www.youtube.com/watch?v=R84_kFXDk9w

5. **Pipette drops**

The dropper used is usually small. Dropper pipettes are useful for transferring water / chemicals from bottles to dripping boards or into tubes.

https://www.youtube.com/watch?v=2cRUOtlt3QU

(Wardiyah , 2016).

6. **P late Drops**
   Board used to mix extracts with reagents in phytochemical screening. Please access the following link : https://www.youtube.com/watch?v=PmDEC4jKZ_A

7. **Ultralight _ Violet**
   this tool used for see chromatogram on TLC. will stain appear form glow or turn off fluorescence when _ subject to long ultraviolet rays (UV -light). waveform (□) 254 or 366 nm. Please access the link https://www.youtube.com/watch?v=3suFcucAlpo

8. **vials**
   Vials are used to accommodate the extraction results in separation by column chromatography.

9. **plate Thin Layer Chromatography (KLT)**
   The TLC plate used for the practicum is a square aluminum TLC plate, measuring: 200 X 200 mm. The TLC plate is made of silica gel of a certain size and is coated on an aluminum plate. This plate is used to separate chemical substances to be identified by the principle of chromatographic separation. Please access : https://www.youtube.com/watch?v=a4DqyhKeU7Q

10. **Vessel Chromatography**
    The chromatography vessel was used as a place to evaluate the TLC plate. The TLC vessel is made of solid glass with no seams at the corners. The vessel has a lid made of corrosion-resistant glass or metal. The vessel must be able to accommodate 200X200 mm plates and be tightly closed. Please access : https://www.youtube.com/watch?v=a4DqyhKeU7Q

11. **Paper strain**
    Filter paper is used to see the saturation of the chromatography vessel

12. **Tweezers**
    Tweezers are used to insert and remove the TLC plates from the chromatography vessel

13. Tube reaction

The test tube is used as a place to react plant extracts with reagents (reagents) in phytochemical screening.

14. A set tool glass

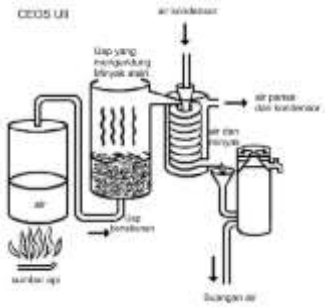
Beaker glass, Erlenmeyer, etc

15. Atomizer _ plate TLC

This tool is used to spray a spot remover on the TLC plate.

16. Distillation stahl

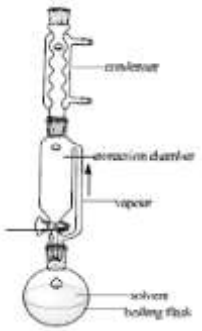
Distillation stahl used for distill ingredients _ _ like leaves and debris wood .



(Julianto , 2019).

17. Soxhlet

Extraction soxhlet only needed if desired compound _ have solubility limited in solvents and impurities no late in solvent it .



(Julianto , 2019).

PRACTICAL MATERIALS

Some of the chemicals used in the phytochemical lab, including:

1. **Methanol , Ethanol , Ethyl Acetate , Chloroform , Water, Hexane , Butanol, Toluene** Solvent this used solvent extractor , or can also be used as component composer phase move on TLC.

2. **Sour chloride (HCl)**
   Hydrochloric acid is used to neutralize bases, provide an acidic environment or to hydrolyze. The hydrochloric acid used for this lab is HCl 2 N and concentrated HCl.

3. **Reactor Wagner**
   Wagner reagent is used to precipitate and detect alkaloids. This reagent was prepared by dissolving 1.27 g I_2 and 2 g KI in water to obtain a volume of 100 mL. Mechanism investigator Wagner can be accessed at the following link : https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

4. **Reactor Mayer**
   This reagent can precipitate alkaloids. Mayer's reagent is prepared by mixing 60 mL of 2.266% w/v $HgCl_2$ with 10 mL of 50% w/v KI solution, then adding water to a volume of 100 mL. With this reagent a precipitate will occur. white . Mechanism investigator mayer can be accessed at the following link : https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

5. **NH_4OH (Ammonia)**
   Pure ammonia vapor is used as a stain remover in the identification of flavonoids with TLC. Meanwhile, dilute ammonia solution is used to provide an alkaline environment to a sample.

6. **Dragendorf**
   Solution dragondorf made with method Mix 20 mL Bismuth nitrate 40% w/v in $_3$p HNO with 50 mL KI 54.4 % w/v. Mixture this then hushed up until split perfect , next taken colored liquid _ clear and diluted with water to a volume of 100 mL. Reactor this used as sighting stains on the identification of alkaloids with TLC, occurred spotting brown / red chocolate . Mechanism investigator Dragendorf can be accessed at the following link : https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

7. **Sour acetate anhydrous**

   $(CH_3.CO)_2O$ solution of the reagent, containing not less 95.0% $C_4H_6O_4$.

8. **Anisaldehyde sulfate**

   Fresh solution obtained by mixing 0.5 mL of Anisaldehyde in 50 mL of glacial acetic acid and 1 mL of H_2SO_4. This solution is used to detect the presence of terpenoids, steroids, and essential oils. This reagent does not last long, do not use it if it has turned red-orange. After spraying on the TLC plate, heat it in the oven at 100°C for 5 -10 minute. Please learn journal on the following link this : https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

9. **Antimony chloride**

   This reagent is used as a stain remover in the identification of terpenoids and steroids which is prepared by dissolving 20 g of antimony chloride in chloroform or ethanol to a volume of 100 mL. Stain observation was carried out after the plate was sprayed and heated for 5-6 minutes at 110°C .

10. **$FeCl_3$**

    Ferric chloride solution is a 10% solution of $FeCl_3$ in water. This solution is used as a stain remover for polyphenol group compounds, a dark purple or blue color will occur old.

11. **KOH**

    Potassium hydroxide dilute (5N) was used as giver atmosphere base while 10% KOH was used as sighting stain on identification compound class anthraquinone

EXPERIMENT 1
IDENTIFICATION OF ALKALOID GROUP COMPOUNDS

Destination

Student knowing method identification compound alkaloid group

Theory Supporters :

Alkaloids are group metabolites secondary found in plants . _ class compound this form mixture from several major alkaloids and minor alkaloids . Alkaloid properties alkaline , contains one or more nitrogen atoms and usually have activity physiology in humans or animal others (Julianto , 2019).

Classification of alkaloids based on the framework the carbon includes :

- True alkaloids (True alkaloids)
  Alkaloids that have framework ring heterocyclic containing a nitrogen atom. Alkaloid biosynthesis types this originated from amino acids . _ Example : Atrophine , Nicotine, Morphine
- Protoalkaloids
  Alkaloids that are not have ring heterocyclic containing nitrogen atoms and is derivative from Amino acids Examples : Ephedrine, mescaline, adrenaline
- Pseudoalkaloids
  these alkaloids contain ring heterocyclic containing nitrogen atoms, however no is derivative from Amino acids Example : Caffeine, theobromine, theophylline

Besides classification above , alkaloids can classified with a number of factor like biosynthesis , skeleton structure chemical , pharmacological , and based taxonomy .

Alkaloids can identified use method thin layer chromatography and spray with a number of alkaloid reagents namely :

- Reactor Dragendorff , results positive give color yellow brown with background behind color yellow from reactor
- Reactor Iodoplatinate , results positive give various colors _
- Marquis reagent , result positive give color yellow until purple
  (Julianto , 2019).
  As ingredient addition theory please learn journal following : https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

Before doing practice please access the following videos :
https://www.youtube.com/watch?v=0GStNN_zgjE

Ingredient

Extract simplicia "X"

How it Works

1. **Setup Sample**

Extract as much as 0.3 grams plus 5 ml of HCl 2 N, heated over a water bath for 2-3 minutes, while stirring. After chilling, add 0.3 grams of NaCl, stir well, then filter. The filtrate obtained was added with 5 ml of 2 N HCl and divided into three parts which were referred to as solutions IA, IB and IC.

2. Reaction Precipitation

IA solution was added with Mayer's reagent, IB solution was added with Wagner's reagent and IC solution was used as a blank. The presence of turbidity or precipitate indicates the presence of alkaloids.

3. Layer Chromatography Thin

IC solution was added with 28% NH_4OH until the solution became alkaline, then extracted with 5 ml of water-free chloroform, then filtered. The filtrate was evaporated to dryness, then dissolved in methanol and ready for examination with TLC.

Phase silent : Kiesel gel GF 254
Phase motion : Ethyl acetate – methanol – water (9 : 2 : 2)
Stain appearance : Reagent Dragendorf
If an orange color appears, it indicates the presence of alkaloids in the extract.

EXPERIMENT 2
IDENTIFICATION OF GLYCOCIDES, SAPONINS AND TERPENOIDS

Destination

Student knowing method identification compound class glycosides , saponins and terpenoids

Theory Supporters :

Glycosides is one _ compound alkaloid type . Alkaloids are compound metabolites secondary on the network plants and animals that have nitrogen atoms (Hartati , 2010). Linking glycosides _ glycones and aglycones it's very easy unraveled by influence acids , bases , enzymes , water, and heat (Rahayu and Hastuti , 2008).

Classification glycosides based on glycone :

- If group glycone something glycosides is glucose so the molecule named as glucoside
- If group glycone something glycosides is fructose so the molecule named as fructose
- If group glycone something glycosides is sour glucuronide so the molecule named as glucuronide and so on .

Classification based on bond glycosides . Based on location bond glycosides , below or above _ from structure flat sugar molecule , then glycosides could classified as alpha - glycosides (below) or beta - glycosides (top).

Based on compound agikon naturally aglycone Glycosides classified as glycosides alcohol , glycosides anthraquinones , glycosides Coumarins , glycosides Chromone , glycoside Cyanogenic , flavonoid glycosides , Glycosides phenolic .

Saponins are compound eliciting active _ foam when shaken in water and at concentration low often cause hemolysis cell blood red (Robinson, 1995). Saponins are glycosides , consisting from bonded sugar groups with aglycone or sapogenins.

Saponins are known two type namely , alcohol triterpenoid glycosides and glycosides certain steroid structures that have chain side spiroketal . Whereas according to structure aglycone or sapogenin, distinguished saponins Becomes type steroids and triterpenoids second _ compound the have connection glycosides and has suggestion the same biogenetics through sour mevalonate and isoprenoid units (Evans, 2002).

Terpenoids are component main in oil essential from a number of type plants and flowers . Compound this in general give strong smell and can _ protect plant from herbivores and predators. Compound terpene Also known as isoprenoid. In nature , compounds terpene dominated as group hydrocarbons , alcohols , glycosides , ethers , aldehydes , ketones , acids carboxylates and their esters . Structure terpenoid compounds are allil cyclic , some among them is compound not fed up with one or more bond double . The consequences compound easy experience reaction addition with hydrogen, halogens, acids and others (Julianto , 2019).

For theory more carry on please learn from the following link : https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/glycoside

https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

Please access :
Glycosides : https://www.youtube.com/watch?v=34DFf0lrnCs
Saponins : https://www.youtube.com/watch?v=cpNEZS-uzic
Terpenoids : https://www.youtube.com/watch?v=RcM7L3h1Evw

Ingredient
Extract simplicia "X"

How it Works

1. **Foam Test**

   0.3 gram of extract was put into a test tube, then added 10 ml of distilled water, shaken vigorously for about 30 seconds. A positive foam test contains saponins if there is a stable foam for more than 30 minutes with a height of 3 cm above the surface of the liquid

2. **Reaction Color**

   0.3 gram of extract is dissolved in 15 ml of ethanol, then divided into three parts of 5 ml each, referred to as solutions IIA, IIB, and IIC

   a. **Liebermann-Burchard test**

   IIA solution was used as a blank, 5 ml of IIB solution was added with 3 drops of anhydrous acetic acid and 1 drop of concentrated H_2SO_4 , then shaken slowly and observed for changes in color. The occurrence of blue green color indicates the presence of steroid saponins, purple red color indicates the presence of steroid triterpenes and light yellow color indicates the presence of saponins. fed up.

   b. Salkowski test

   IIA solution was used as a blank, 5 ml of IIC solution was added 1 – 2 ml of concentrated H_2SO_4 through the test tube wall. The presence of unsaturated steroids is indicated by the appearance of a red ring.

3. **Identification of steroidal or triterpenoid sapogenins TLC**

   Extract as much as 0.5 grams plus 5 ml of 2 N HCL, boiled and covered with a funnel containing wet cotton for 2 hours to hydrolyze saponins. After cooling, neutralize with ammonia, then extract with 3 ml of n-hexane 3 times, then steam until only 0.5 ml remains, dab on the plate TLC.

   Phase silent : Kiesel Gel GF 254
   Phase motion : n-hexane-ethyl acetate (4:1) Spot
   remover : - Sulfuric acid anisaldehyde
   - Antimony chloride

   The presence of sapogenins is indicated by the appearance of a color :
   - purple red (purple) for acid anisaldehyde sulfate

- pink for antimony chloride

4. Identification of free terpenoids or steroids in a manner TLC

A little extract added a few drops of ethanol, stirred until dissolved, dappled on the stationary phase. This thin layer chromatography test uses:

Phase silent : Kiesel gel GF 254
Phase motion : n-hexane–ethyl acetate (4 : 1)
Stain appearance : Acid anisaldehyde sulfate

The presence of terpenoids or steroids is indicated with happening color red purple or purple

EXPERIMENT III
IDENTIFICATION OF FLAVONOID COMPOUNDS

Destination
Student knowing method identification compound the flavonoid group

Theory Supporters :
Flavonoids are one _ class compound phenol greatest nature _ in plant . and is composed of 15 carbon atoms as the core . Flavonoids are found in plants , which contribute produce colored pigments yellow , red , orange , blue , and colors purple from fruits , flowers and leaves . Flavonoids included in family soluble polyphenols _ in water.

Flavonoids are polar compounds , then the flavonoids will late good in polar solvents like ethanol , methanol , butanol, acetone , dimethylformamide and others . Because of bound flavonoids in form glycosides so mixture solvent the on with water is good solvent _ for flavonoid glycosides . Whereas in form aglycone like flavones , flavonols , flavanones more easy late in solvent chloroform and ether .

There are several subclass of flavonoids: flavanols, flavanones , flavones , isoflavones , anthocyanidins, and flavonols . Distribution in Flavonoid subclasses are based on these properties structural . Flavanols found in Grape red and wine red (ex-catechins), flavanones found in food citrus (ex -narigenin), flavones (exapigenin) were found in spice leafy green , isoflavones found in food soy , and at nearly all food flavonols found . Origin of flavonoids catechins especially found in tea green and black and grapes red , meanwhile anthocyanin found in strawberries and berries berries others , wine , wine and tea (Arifin and Ibrahim, 2018).
Please learn more carry on about flavonoids through journal following :
https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

Please access :
https://www.youtube.com/watch?v=MW9dcAzAoew
https://www.youtube.com/watch?v=JYrMxfvj-8Y

Ingredient
Extract simplicia "X"

How it Works

1. **Reaction Color**

   0.3 gram of extract was shaken with 3 ml of n-hexane many times until the n-hexane extract was colorless. The residue was dissolved in ethanol and divided into 4 portions, each referred to as IIIA, IIIB, IIIC, and IIID solution.

   a. **Bate-Smith test and Metcalf**

Solution IIIA was used as a blank, solution IIIB was added with 0.5 ml of concentrated HCl and observed for the color change that occurred, then heated over a water bath and observed again for the color change that occurred. If it slowly turns bright red or purple, it indicates the

presence of leukoanthocyanin compounds (compared to blank). Wilstater and Bate-Smith test mechanism please learn journal this https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

b. Wilstater test

Solution IIIA as a blank. IIIC solution added 0.5 ml of concentrated HCl and 4 pieces of magnesium. Observe the color that occurs. Diluted with distilled water, then added 1ml butanol. Observe the color that occurs in each layer. A change in orange red color indicates the presence of flavones, pale red indicates the presence of flavonols, dark red indicates the presence of flavonones.

2. Layer Chromatography

The IIID solution is dotted on the stationary phase. This thin layer chromatography test uses:
Phase silent : thin layer cellulose
(replaced by Kiesel Gel GF 254)
Phase motion : butanol-glacial acetic acid-water (4 : 1 : 5)
Stain appearance : - boric citrate reagent or
- ammonia vapor
The presence of flavonoids is indicated by the appearance of intense yellow stains.

The yellow stain caused by ammonia vapor will disappear slowly when the ammonia evaporates leaving the stain. Meanwhile, the yellow stain caused by citrate borate reagent is permanent.

The mobile phase is commonly called **BAW** (butanol, Acetic acid, Water). BAW is made by mixing these three components. With the ratio B : A : W = 4 : 1 : 5, there will be 2 layers. The top layer was taken and used as the mobile phase to evaluate the compound group flavonoids .

EXPERIMENT IV
IDENTIFICATION OF POLYPHENOL AND TANIN COMPOUNDS

Destination

Student knowing method identification compound class polyphenols and tannins

Theory Supporters :

Polyphenols is one _ category biggest from most of the phytochemicals its spread among the plant kingdom . Compound Phenolic is compounds that have one or more group hydroxyl attached to the ring aromatic .

Compound polyphenols known as antioxidants experience because produce activity antioxidants , play a role as agent reducing and antioxidant hydrogen atom donor . Polyphenols could hinder , prevent , reduce oxidation by radicals free so that good for health (Nely , 2000).

tannins is something compound phenolic which gives a bitter and astringent/ chelating taste , can react and agglutinate the protein or other organic compounds that contain amino acids and alkaloids. Compound this role important for protect plant from predation by herbivores and pests , as well as as agent regulator in metabolism plant .

tannins grouped Becomes two form compound namely :

- tannins Hydrolyzed tannins in form this are acid hydrolyzed tannins _ or enzyme produce sour gall and acid elagat . kindly chemically , hydrolyzed tannins could is an ester or sour phenolic .
- Condensed tannins tannins type this resistant to reaction hydrolysis and usually lowered from compound flavonols , catechins , and flavan-3,4-diol. On additions sour or enzymes , compounds this will decomposed Becomes plobapen
  (Julianto , 2019).

Please understand journal following this through links : https://drive.google.com/drive/folders/1MyAEs6AXSmQgTg0FLwn8ZqZBz4ctWD5N?usp=sharing

Please access :
https://www.youtube.com/watch?v=J8TTFhJdQd8

Ingredient

Extract simplicia "X"

How it Works

1. **Reaction Color**

   0.3 gram of extract plus 10 ml of hot distilled water, stirred and left to room temperature, then added 3-4 drops of 10% NaCl, stirred, and filtered. The filtrate is divided into three parts @ 4 ml each and is referred to as IVA, IVB and IVC solutions.

a. Ferrichloride Test

A few drops of FeCL3 solution were added to the IVC solution , then the color change was observed. If a blackish green color occurs, it indicates the presence of tannins. If no precipitate appears on the addition of gelatin and NaCl but after adding FeCl3 solution , the color changes to blue green to black, indicating the presence of polyphenolic compounds.

$FeCl_3$ positive, test gelatin positive ⟶ tannin (+)

$FeCl_3$ positive, test gelatin negative ⟶ polyphenol (+)

$FeCl_3$ negative ⟶ polyphenols (-), tannins (-)

b. Gelatin Test

IVA solution was used as a blank, IVB solution was added with a small amount of gelatin solution and 5 ml of 10% NaCl solution. If a white precipitate occurs, it indicates the presence of tannins .

2. Layer Chromatography

Part of the IVA solution is used for examination with TLC.

phase : Kiesel gel GF 254

Phase motion : Chloroform – ethyl acetate (1 : 9)

Spot remover: Reagent $FeCl_3$

If a black color appears, it indicates the presence of polyphenols in the sample .

EXPERIMENT V
IDENTIFICATION OF ANTHRACHINE COMPOUNDS

Destination

Student knowing method identification compound class anthraquinone

Theory Supporters :

Anthraquinone is class from compound glycosides including derivative quinone (Sirait , 2007). Anthraquinone is compound crystal dotted melt high and dissolved in solvent organic and alkaline . Anthraquinone easy hydrolyzed . Compound anthraquinones and their derivatives often colored yellow until red sindur (Setyawaty *et al.,* 2014) .

Anthraquinone could bond with sugar as oglycosides or as a c- glycoside . Derivative anthraquinone generally late in hot water or in alcohol watery . Compound anthraquinone could react with base give color yellow until red as well as purple or green (Lantriyadi , 2017).

Anthraquinone many used in the field industry and medicine for various necessity . Anthraquinone show activity interesting in vitro biology like antimicrobial , hypotensive and antilukemic . Component anthraquinone is molecules that are clinical considered important especially rubiadin , damnacanthal , alizarin, and purpurin were used in drug formulas chemotherapy (Asrinah , 2017).

Please access :

https://www.youtube.com/watch?v=8qI1595Q2pk

Ingredient

Extract simplicia "X"

How it Works

1. **Reaction Color**
   a. **Borntrager test**

      0.3 gram of extract was extracted with 10 ml of distilled water, filtered, then the filtrate was extracted with 3 ml of toluene in a separatory funnel. Extraction was carried out twice. Then the toluene phase is collected and divided into two portions, referred to as VA and VB solutions. VA solution as a blank. VB solution added with ammonia and shaken. Red color indicates the presence of anthraquinone compounds.

   b. **Modification Test Borntrager**

      $_{1}$ ml of 5N KOH and 1 ml of $_{dilute}$ H_2SO_4 . Heated and filtered, the filtrate was added with glacial acetic acid, then extracted with toluene. The toluene phase was taken and divided into two as VIA and VIB solutions. VIA solution as blank, VIB solution plus ammonia. A red or pink color in the alkaline layer indicates the presence of anthraquinones

2. Layer Chromatography

The sample was spotted on the stationary phase under the following thin layer chromatography conditions:

Phase silent : Kiesel gel GF 254
Phase motion : toluene – ethyl – acetic acid (75 : 24 : 1) Stain
appearance : 10% KOH solution in methanol.

The appearance of stains yellow, yellow brown, red purple or green purple indicating the presence of anthraquinones .

EXPERIMENT VI
EXTRACT PRODUCTION BY MACERATION

Destination

Student knowing method making extract vegetable with maceration

Theory Supporters :

Extraction is a separation process something substance from the mix with use solvent , solvent used _ must could extract desired substance _ without dissolve other materials . Destination extraction ingredient natural is for interesting component chemical in the material _ nature .

Broadly speaking , the separation process in a manner extraction consists from three step base namely :

- Addition a number mass solvent for contacted with sample , usually through the diffusion process .
- Substance dissolved will separated from sample and dissolved by the solvent form phase extract .
- Separation phase extract with sample
  (Wilson *et al* ., 2000).

Extract is preparations the concentration obtained with method extract substance active with use suitable solvent , then _ all or almost all solvent evaporated and mass or remaining powder _ treated such that , until Fulfill established standard (Ministry of Health RI 1995).

Maceration is method extraction with use stationary solvent or with exists stirring several times at temperature room . method this could conducted with method soak ingredient with once in a while conducted stirring . By and large immersion conducted for 24 hours, then solvent replaced with solvent new . Maceration can also conducted with stirring in a manner continuous (maceration kinetic). Advantages from method this that is effective for compounds that are not stand heat (degraded because heat), the equipment used relatively simple , cheap , and easy got . However method it also has a number of weakness that is time long extraction , requires solvent in large number , and existence possibility that compound certain no could extracted because low solubility at temperature _ space (Sarker et al., 2006).

Please access :

https://www.youtube.com/watch?v=qrc4Ru2HWdk

Ingredient

Simplisia *Curcuma xanthorrhyza*

How it Works

As much as 500 grams of dry powder was put into the macerator, 96% ethanol was added as much as 7 ½ times the weight of the powder and stirred. Let it macerate for 5 days in a closed macerator with daily stirring. After that, filter the macerate from the dregs with a

Buchner funnel . Maserate is deposited for 2 days. After that, carefully separate the macerate from the enapan. Evaporate the macerate in a porcelain cup over a water bath or with a *rotary evaporator (rotavapour)* to obtain a thick extract.

The thick extract obtained was precipitated by adding excess ethanol. And kept for 2 days. Then it is extracted with the help of a suction pipe .

EXPERIMENT VII
EXTRACT PRODUCTION BY PERCOLATION

Destination
Student knowing method making extract vegetable with percolation

Theory Supporters :
Extract is preparations the concentration obtained with method extract substance active with use suitable solvent , then _ all or almost all solvent evaporated and mass or remaining powder _ treated such that , until Fulfill established standard (Ministry of Health RI 1995).

By and large extraction conducted with use solvent based on solubility _ component to other components inside mixture , usually water and something else solvent organic . material to be extracted usually form ingredient dry that has been destroyed , usually shaped powder or simplisia (Sembiring , 2007).

Percolation is method extraction with prepared material _ in a manner bonfire with use always solvent _ new until the process is a perfect 10 and generally conducted on temperature room . Procedure method this that is ingredient soaked with solvent , then solvent new streamed in a manner Keep going continuously until color solvent no again colored or permanent clear which means already no there is again dissolved compounds . _ Advantages from method this that is no additional processing is required for separate solids with extract , meanwhile weakness method this is amount the required solvent enough many and the process also requires quite a long time, as well no evenly contact Among solids with solvent (Sarker *et al* ., 2006).
Please access : https://www.youtube.com/watch?v=qrc4Ru2HWdk

Ingredient
Simplisia *Curcuma xanthorrhyza*

How it Works

Wetting: As much as 500 grams of dry matter powder is added to 1/2 to equal amount of 96% ethanol by weight of the powder, little by little while stirring carefully. Let it soak for 2 hours.

Percolation : The bottom of the percolator is filled with cotton and then filter paper is put on it. Add the ingredients that have been moistened and add the liquid extract until approximately ¾ of the percolator. Let it macerate overnight. The next day the percolator faucet was opened by adjusting the percolator flow rate. Perkolate is accommodated in the container provided. Monitor the filter liquid above the powder in the percolator, if it almost reaches the surface of the powder, add more filter liquid. Percolation was continued until the liquid above the powder was clear. The obtained percolate was concentrated with a rotary evaporator (rotavapour) to become an extract.

The thick extract obtained was precipitated by adding excess ethanol. And kept for 2 days. Then it is extracted with the help of a suction pipe .

EXPERIMENT VIII
FRACTIONATION WITH COLUMN Chromatography

Destination

Student capable To do fractionation extract plant with chromatography column

Theory Supporters :

Fractionation in principle is the withdrawal process compound in one extract with use two type no solvent _ each other mixed . common solvent _ worn for fractionation is n -hexane , ethyl acetate , and methanol . For attracts fats and non-polar compounds are used nhexane , ethyl acetate for interesting semi-polar compounds , while methanol for interesting polar compounds . from this process could suspected nature polarity from compounds to be separated . as is known that non - polar compounds _ late in non-polar solvent whereas polar compounds _ _ _ late in polar solvents as well (Mutiasari , 2012).

one _ method fractionation separation in a manner chromatography is liquid vacuum chromatography or vacuum liquid chromatography (VLC). VLC is column chromatography _ _ _ with use vacuum for speed up Genre eluent .

Principle work from VLC is exists adsorption or absorption , meanwhile separation based on the compounds to be separated distributed among _ stationary phase and phase motion in different comparisons . _ Phase motion with gradient polarity expected capable separate compounds with different polarity (Sastrohamidjojo , 2005).

On VLC, column packed dry in circumstances vacuum to obtain density absorbent (eg silica gel) maximum . Sample made powder together with absorbent (impregnation) and put in got on column then sucked slowly use vacuum . next column eluted use suitable solvent , is started with nonpolar solvent . Column in vacuum until dry on each collection fraction . vacuum discontinued when dry and column could used return if column no cracked or descent eluent already level with column (Raymond, 2006).

Please access :

https://www.youtube.com/watch?v=AZQITgeJcYA

Material :

Extract *Curcuma xanthorrhyza*

How it works :

1. **Washing Extract**

   The extracts obtained from exercises VII & VIII were put in a beaker and added hot 96% ethanol. Allow it to cool to 50°C, then filter it with a Buchner funnel until there is no dripping ethanol. The above procedure was repeated until a curcuminoid extract was obtained which was free of impurities (indicated by a change in the color of the extract to a brighter one). The extract obtained was then aerated in a fume hood to dry. The dry extract was then fractionated to obtain curcumin isolate.

2. Election Eluent for Fractionation

Standard curcumin and curcuminoid extract which had been washed were dissolved in 96% ethanol and 2-5 µl was applied to the TLC plate. The TLC plate was then eluted using the appropriate eluent in the chromatography bath up to the specified limit. Observe the plates in a UV lamp at 254 nm and 365 nm. The eluent was chosen if the curcuminoid extract that was bottled was separated into 3 stains namely curcumin, bisdemethoxy curcumin and desmethoxy curcumin. See the picture below

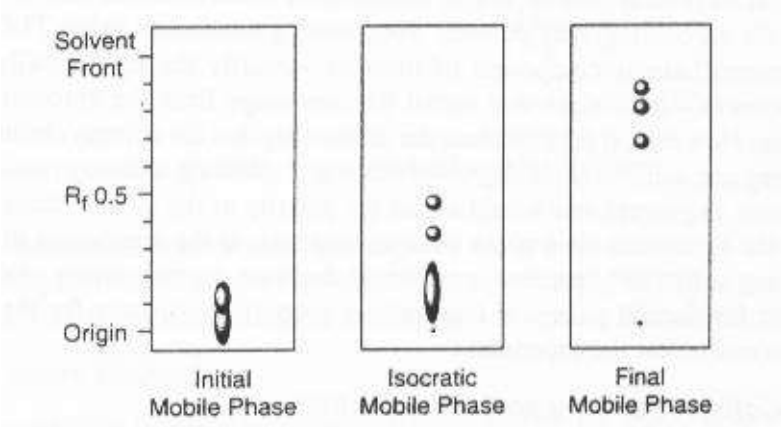


Figure 4. TLC plate with extract curcuminoids

3. Fractionation with Chromatography Column

The steps for fractionation by column chromatography are as follows:

a. Silica gel as much as 100 times its weight extract curcuminoids entered in Erlenmeyer and added with eluent □ 2 cm above surface silica gel, shaken slow until evenly and enter with careful to in column chromatography which is in section underneath has given *glass wool* . the column then hushed up for 1 day for compress and view there is nope cracks (see picture under this).

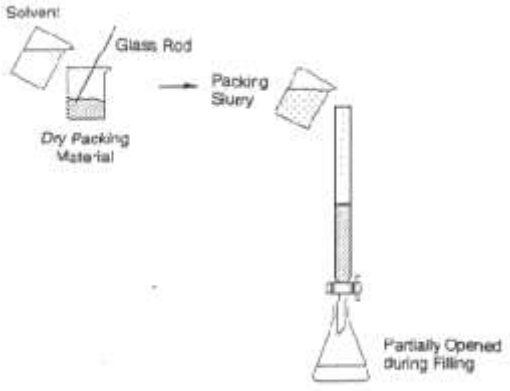


Figure 5. Separation steps by column chromatography

b. If the column is not cracked, add 0.5 cm of eluent above the surface of the silica gel and if it is cracked repeat step a. Then into the column added curcuminoid extract

(1% by weight of silica) which has been mixed with silica gel.

c. Stream eluent and contain □ 50 ml in Erlenmeyer (eluent this not yet bring substance chemical plant so that could discarded). Then the faucet is opened and adjusted drops (1 drop/ second) and collected in vials or tube that has given number of each 5 ml vial (see picture under this).

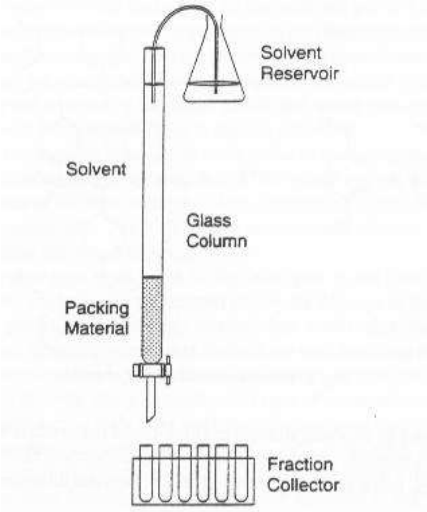


Figure 6. System chromatography column base

d. In each vial with a multiple of 10, a TLC test was carried out to see the stains produced. If they produce the same stain, the vials are combined. Dropping was stopped when the vial was not stained when tested TLC .

EXPERIMENT IX
ISOLATION OF FLAVONOID GLYCOCIDES FROM PASSAS LEAVES

Destination

At the end of the practicum, students are expected to be able to understand and be able to isolate flavonoids from cassava leaves along with a qualitative analysis of these compound groups using the thin layer chromatography method.

Supporting Theory :

Flavonoids are present in all plant green so that could found on each extract plant . Flavonoids are found in plants , which contribute produce colored pigments yellow , red , orange , blue , and colors purple from fruit , flowers and leaves (Arifin & Ibrahim, 2018). Most _ flavonoid compounds in plant found in form glycosides which means that the flavonoid units are bound to a sugar. Flavonoid glycosides are formed because group hydroxyl in Flavonoid molecules (aglycones) bind together with group carbonyl of sugars (glycones). In plants , these flavonoid glycosides have function as sugar reserves because he no could transported by cells plant because exists part aglycone (group other than sugar) (Sani *et al* ., 2013).

According to Harborne (1987) that flavonoids in plants Often present as glycosides (flavonoids). glycosides) and are rarely found in single form/ aglycone of flavonoids, therefore it is to analyze the flavonoids better to hydrolyze the bound glycosides on flavonoids the before attention to the complexity of the glycosides may be present in the original extract .
please : https://www.youtube.com/watch?v=46meC5v7alU

Material :

Simplisia *Manihot utilissima* Pohl

How it works :

a. Isolation :

Weigh 40 grams of the powdered material, put it in an infusion pot and add 240 ml of water. Simmer for 30 minutes. Filter the mixture through a Buchner funnel to obtain a clear filtrate and transfer it to a clean 250 ml Erlenmeyer. Store in the refrigerator for 1 week until yellowish-white amorphous crystals form. Pour most of the clear solution carefully so that the crystals do not pour out, then filter the crystals that are on the Erlenmeyer bottom through the tared filter paper. If there are still crystals attached to the bottom of the Erlenmeyer, rinse with distilled water and pour the rinse onto filter paper, wash the crystals with 10 ml of ice water. Dry the filter paper with the precipitate at 50 ˚C, until dry then weigh to obtain the yield of the results got.

b. Identification

Take a small amount of solid with the tip of a small spatula, dissolve it in 2 ml of a mixture of methanol and water, vortex until dissolved. The solution was ready to be analyzed

qualitatively by thin layer chromatography under the following conditions:

a. Phase silent : Silica gel GF 254
   Phase motion : sour acetate 15%
b. Sample : solution sample and comparison solution routine in 50% methanol each as much as 10 stupid
c. Detection : Vapor ammonia , below ray visible and UV 366

Every time detect , mark spotting visible flavonoids _ with a dot next _ right and left . Note Rf prices and formed color . _

EXPERIMENT X
IDENTIFICATION OF ETHYL- *p* -METHOXY CINAMAT FROM KENCUR

Destination

Student capable explain principle basics and techniques isolation , as well capable To do separation and purification results isolation from ingredient plant

Theory Supporters :

Ethyl p -methoxycinnamate is one _ product nature found in kencur (Kaempferia galanga Linn.) relative amount _ big . Ethyl p -Methoxycinnamate (EPMS) incl in class ester containing compounds ring benzene and groups nonpolar methoxy as well as groups bonding carbonyl _ characteristic ethyl _ slightly polar so in the extraction could use the solvents they have variation polarity that is ethanol , ethyl acetate , methanol , water, and hexane (Setyawan *et al* ., 2012). Ethyl p -methoxycinnamate have group reactive function _ so that could transformed Becomes group other functions , among others is bond double conjugated , ring group - activated aromatics _ methoxy and ester groups (Taufikurohmah *et al* ., 2008).

Please access : https://www.youtube.com/watch?v=a7dBmmou4Gg

Ingredient

Kencur rhizomes (*Kaemferiae rhizoma*)

How it Works

1. **Isolation ethyl *p* - methoxycinnamate**

   Put about 15-20 g of kencur powder into a 250 mL round flask, then add 100 mL of n-hexane. Attach the reflux condenser to the circular flask and reflux in the water bath for 60 minutes. Filter the refluxed kencur mixture and perform a simple distillation of the filtrate in the circular flask in a water bath until about 10 mL of solution remains in the flask. Cool the pumpkin to room temperature until white crystals form. If no crystals form, chill the pumpkin in a bowl of ice. Filter the white crystalline solid formed, weigh the crystals and calculate the yield. Recrystallization is carried out in petroleum ether or n-hexane, then the melting point is measured (Lit. 48-50oC) .
2. **Hydrolysis ethyl - *p* - methoxy cinnamic**

   As much as 2.5 g of ethyl - *p* -methoxycinnamate was dissolved in 5 mL of ethanol in a 100 mL round flask. Add 1.25 g of NaOH and 20 mL of water, the reaction mixture is refluxed for 30 minutes then cooled to room temperature. Neutralize with dilute HCl to produce white crystals, filter with a Buchner funnel and the crystals obtained are washed with water. Recrystallization was carried out with methanol solvent. measure the melting point and compare it with the literature (Lit. 174 ◦C).
3. **Inspection Thin Layer Chromatography (KLT)**

Sample crystal results isolation and yield hydrolyzed respectively dissolved in n - hexane , using a capillary tube spotted on the plate TLC size 2 x 5 cm, at a distance of 0.5 cm from down . Input in the chamber that has saturated with eluent chloroform:n -hexane = 9:1. Observation spotting conducted with see apparition stain below _ UV lamp . Calculate the Rf of that compound obtained .

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